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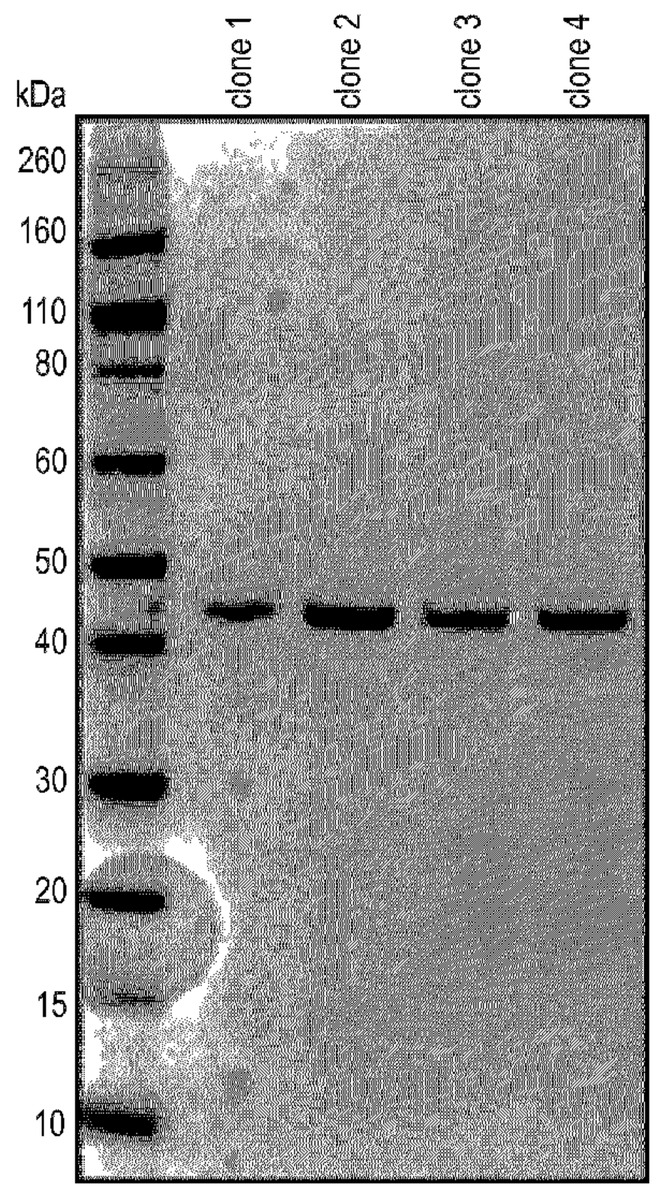
(71) Demandeur/Applicant:
GENOVIS AB, SE

(72) Inventeurs/Inventors:
LEO, FREDRIK, SE;
LOOD, ROLF, SE;
BJORK, STEPHAN, SE;
MEJARE, MALIN, SE;
OLSSON, FREDRIK, SE

(74) Agent: BLAKE, CASSELS & GRAYDON LLP

(54) Titre : PROTEASE ET POLYPEPTIDE DE LIAISON POUR O-GLYCOPROTEINES
(54) Title: PROTEASE AND BINDING POLYPEPTIDE FOR O-GLYCOPROTEINS

Fig. 1



(57) Abrégé/Abstract:
The present invention relates to a novel endoprotease, mutants thereof having binding but lacking or having reduced hydrolyzing activity, and use in methods of studying and isolating O-linked glycoproteins.

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(71) Applicant: **GENOVIS AB** [SE/SE]; P.O. Box 790, 220 07 Lund (SE).

(72) Inventors: **LEO, Fredrik**; c/o Genovis AB, P.O. Box 790, 220 07 Lund (SE). **LOOD, Rolf**; c/o Genovis AB, P.O. Box 790, 220 07 Lund (SE). **BJORK, Stephan**; c/o Genovis AB, P.O. Box 790, 220 07 Lund (SE). **MEJARE, Malin**; c/o Genovis AB, P.O. Box 790, 220 07 Lund (SE). **OLSSON, Fredrik**; c/o Genovis AB, P.O. Box 790, 220 07 Lund (SE).

(74) Agent: **LEWIS, Graham**; J A Kemp, 14 South Square, Gray's Inn, London Greater London WC1R 5JJ (GB).

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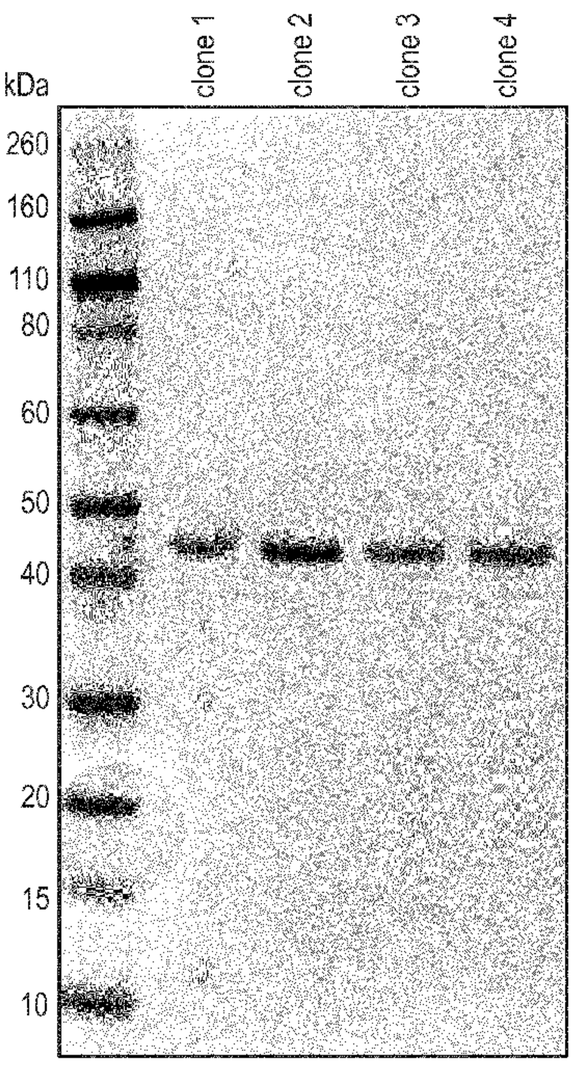

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CLAIMS

1. A polypeptide having endoprotease activity specific for O-glycosylated proteins which comprises:

(a) an amino acid sequence of SEQ ID NO: 1;

(b) an amino acid sequence which is at least 85% identical to the amino acid sequence of SEQ ID NO: 1 or

(c) an amino acid sequence which is a fragment of the sequence of SEQ ID NO: 1 or a fragment of an amino acid sequence which is 85% identical to the amino acid sequence of SEQ ID NO: 1.

2. The polypeptide according to claim 1, wherein the amino acid sequence which is at least 85% identical to the amino acid sequence of SEQ ID NO: 1, or the fragment thereof, comprises the motif HEbbH, wherein b is an uncharged amino acid, optionally A, C, F, G, I, L, M, N, P, Q, S, T, V or W, and optionally wherein said motif is present in said polypeptide at positions corresponding to positions 181 to 185 of SEQ ID NO: 1.

3. The polypeptide according to claim 2, wherein said motif comprises the sequence HEIGH or HELGH, preferably HELGH.

4. The polypeptide according to any one of the preceding claims which comprises the motif abxHEbbHbc, wherein:

(a) a is amino acid V, T or G;

(b) b is an uncharged amino acid, optionally A, C, F, G, I, L, M, N, P, Q, S, T, V or W;

(c) x is any amino acid; and

(d) c is a hydrophobic amino acid, optionally A, C, F, I, L, M, P, V, W or Y.

optionally wherein said motif comprises the sequence GMAHELGHGL or GVAHELGHNF, preferably GMAHELGHGL.

5. The polypeptide according to any one of the preceding claims, which includes an additional methionine at the N terminus and/or a His tag at the C terminus, which tag may

be joined to the C terminus by a linker, optionally wherein said polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 2.

6. The polypeptide according to any one of claims 1 to 4, wherein the polypeptide is provided in solution, lyophilised, or immobilised, optionally wherein the polypeptide is provided together with a sialidase, preferably Am1757 or a mixture of Am1757 and Am0707, wherein Am1757 is a polypeptide consisting of SEQ ID NO: 11 and/or wherein Am0707 is a polypeptide consisting of SEQ ID NO: 14.

7. A method of hydrolysing an O-glycoprotein, wherein the method comprises contacting a sample comprising the protein with a polypeptide according to any one of claims 1 to 6 and optionally further comprising the detection or analysis of the hydrolysis products.

8. A method for assessing the glycosylation status of a protein, comprising contacting a sample comprising the protein with a polypeptide according to any one of claims 1 to 6 and detecting and/or analysing the products produced, optionally wherein the presence or absence of cleavage products is used to determine the presence or absence of an O-glycoprotein in the sample, and/or wherein said analysis is conducted to identify the type of a O-glycan chain and/or its position of attachment to an O-glycoprotein.

9. The method according to claim 7 or 8, wherein the analysis or detection is carried out by affinity chromatography, SDS-PAGE, HPLC or mass spectrometry.

10. The method according to any one of claims 7 to 9, wherein the sample is incubated with a sialidase prior to or at the same time as contacting the protein or sample with the polypeptide of claims 1 to 6, preferably wherein said sialidase is Am1757 or a mixture of Am1757 and Am0707.

11. The method according to claim 10, wherein Am1757 is a polypeptide consisting of SEQ ID NO: 11 and/or wherein Am0707 is a polypeptide consisting of SEQ ID NO: 14.

12. A polypeptide which is capable of binding to an O-glycan or O-glycoprotein and which lacks or has reduced endoprotease activity specific for O-glycosylated proteins comprising:

(a) an amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 20;

(b) an amino acid sequence which is at least 85% identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 20; or

(c) an amino acid sequence which is a fragment of the sequence of SEQ ID NO: 5 or SEQ ID NO: 20 or a fragment of an amino acid which is 85% identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO:20.

13. The polypeptide according to claim 12, wherein the amino acid sequence which is at least 85% identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 20, or the fragment thereof, does not comprise the metallprotease motif HEbbH and preferably comprises a disrupted version of said motif, such that:

(a) H in the first position is replaced with an alternative amino acid, preferably A or G; and/or

(b) E in the second position is replaced with an uncharged amino acid, optionally A, C, F, G, I, L, M, N, P, Q, S, T, V or W, preferably A or G; and/or

(c) H in the fifth position is replaced with an alternative amino acid, preferably A or G

wherein b in the said motif is an uncharged amino acid, optionally A, C, F, G, I, L, M, N, P, Q, S, T, V or W.

14. The polypeptide according to claim 12 or 13, which includes an additional methionine at the N terminus and/or a His tag at the C terminus, which tag may be joined to the C terminus by a linker, optionally wherein said polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 6 or 21.

15. The polypeptide according to any one of claims 12 to 14, wherein the polypeptide is provided in solution, lyophilised, or immobilised, optionally wherein the polypeptide is

provided together with a sialidase, preferably Am1757 or a mixture of Am1757 and Am0707.

16. A method of binding to an O-glycan, O-glycopeptide and/or O-glycoprotein wherein the method comprises contacting a sample comprising the O-glycan, O-glycopeptide and/or O-glycoprotein with a polypeptide according to any one of claims 12 to 15, and optionally determining whether or not an O-glycan, O-glycopeptide and/or O-glycoprotein has been bound and/or separating the O-glycan and any linked glycoprotein, the O-glycopeptide or O-glycoprotein from the resulting mixture; optionally wherein the method is for the purpose of isolating an O-glycan or linked glycoprotein, O-glycopeptide or O-glycoprotein from the sample.

17. A method for assessing the glycosylation status of a protein, comprising contacting a sample comprising the protein with a polypeptide according to any one of claims 12 to 15 and determining whether or not the protein is bound by the said polypeptide.

18. A method for detecting O-glycopeptides and/or O-glycoproteins in a sample, wherein the method comprises:

(a) contacting said sample with a polypeptide according to any one of claims 12 to 15 to thereby allow formation of complex between an O-glycopeptide and/or O-glycoprotein and the said polypeptide;

(b) optionally separating said polypeptide from the contacted sample; and

(c) determining whether the separated polypeptide is bound to an O-linked glycoprotein or glycopeptide, thereby determining the presence or absence of O-linked glycopeptides or glycoproteins in the sample.

19. The method according to any one of claims 16 to 18, wherein said determining and/or separating is carried out by affinity chromatography, SDS-PAGE, HPLC, lectin blotting, ELISA or mass spectrometry.

20. The method according to any one of claims 16 to 19 which additionally comprises a step of eluting bound material from the polypeptide of any one of claims 12 to 15 with a buffer comprising:

(a) High molar concentration Urea;

(b) High concentration detergent; or

(c) A polypeptide according to any one of claims 1 to 5.